

Microwave Dielectric properties of Osteosarcoma Cell Line (SAOS-2) Suspensions

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Abstract—Dielectric properties of biological tissues have been limited to some in vivo animal and mostly ex vivo animal and human tissue measurements. Recent studies showed that factors changing after the excision of tissue such as temperature, heterogeneity, hydration can significantly alter the dielectric properties. However, due to the challenging nature of performing dielectric properties on living tissues, the underlying factors of dielectric property discrepancy between diseased and healthy tissues remains an open question. As building blocks of tissues, cells can serve as an alternative sample to living tissue to explore the fundamental dielectric property discrepancy between normal and diseased tissues. With this motivation, we are reporting two different concentrations of Osteosarcoma Cell Line (SAOS-2) suspended in Dulbecco's Modified Eagle Medium (DMEM) dielectric property measurements. Dielectric property measurements are performed with the open-ended coaxial probe technique between 500 MHz to 18 GHz. A very small decrease (0.5 units) is observed between the relative permittivity of pure DMEM and cell suspension. Similarly, the difference between dielectric properties of 12,500,000 SAOS-2 cell suspensions and 22,500,000 SAOS-2 cell suspensions is found to be small and inconsistent at frequencies between 500 MHz to 18 GHz.

Index Terms—cell suspension, dielectric properties, open-ended coaxial probes, SAOS-2

I. INTRODUCTION

Reported cell dielectric properties in microwave region have been limited with few studies and these properties have been mostly investigated at low frequency (LF) to very high frequency (VHF) region; that is, ranging from 30 kHz to 300 MHz [1], [2]. To measure the dielectric properties of cells, several methods can be performed such as dielectrophoresis, resonant cavity perturbation technique, and the open-ended coaxial probe method. Dielectrophoresis technique is based on the principle of the motion of the particles due to their polarization when applied a non-uniform electric field in order to research their dielectric properties. In [3], the authors focused on the dielectric properties of mouse ovarian surface epithelial cells. In this study, contact-less dielectrophoresis technique has been applied to the cells which are at different stages (early, early intermediate, intermediate, late) [4]. They have found that malignant and benign cells are differentiated

in metabolism, proliferation cytoskeleton and also some other functional properties [5]. These differences cause distinct dielectric properties between these type of cells. Among these studies, only few measurements are reported in microwave region.

Research on dielectric properties of biological tissues have been of importance to the progress of research in microwave diagnostic and therapeutic technologies. Reported studies are first concentrated on animal tissues then measurement on ex vivo human tissues are conducted. In [6], ultra wideband dielectric property measurements of normal ex vivo human tissues and the tissues are classified based on its water content. This study are then followed by several other publications reporting the dielectric properties of normal and diseased tissues to collect the necessary dielectric property knowledge for development of microwave therapeutic and diagnostic technologies. Most of these studies are performed with the open-ended coaxial probe method, since this method is non-destructive and provides broadband measurements. Few cell dielectric properties have also been measured with the open-ended coaxial probe technique at the microwave region [7], [8]. These studies handle the cell samples mostly in three different forms; that is, pellet, cell suspension, cells in gel medium. Pellet form is the bulk form of cells obtained after the centrifugation process. to compose the cell suspension, the cells are dispersed in a culture medium and to compose the gel form, the cells are embedded in matrix to a gel medium. Mostly the reported research focusses on the cell suspensions rather than pellet measurements. Measured dielectric properties of cells are then retrieved by using mixture equations. There is still a need to form a consensus on performing dielectric property measurements on cells.

To this end, we are using cell suspensions with different cell concentrations to understand the dielectric properties of the cells at microwave region and to establish a measurement consensus for measurement of cell dielectric properties with the open-ended coaxial probes. In this work, dielectric properties of pure DMEM as well as 12,500,000 and 22,500,000 cell suspensions are measured with the open-ended coaxial probe technique. The measurement set-up is discussed in Section II-A, the sample preparation is explained in II-B, and the results are given in III.

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II. METHODOLOGY

A. Measurement Set-up

The device used in this study is Agilent N5230A PNA-L Network Analyzer. The probe used in the study Agilent (now Keysight) Slim Form Probe. The other materials that are used in the study are a laptop and Agilent 85070E software. The dielectric property measurement set-up and the measurement sample are shown in Fig. 1a and Fig. 1b, respectively. Before the measurements, the device needs a calibration to obtain accurate results. It should be done carefully as it is suggested in software instruction. In general, the open-ended coaxial probe use three common standards for calibration: Open air, distilled water (or any liquid, dielectric properties of which is known) and conductive textile (short). Since environmental changes may affect measurements results, temperature, humidity and pressure should be monitored [9]. Probe tip should be cleaned, and cable should be fixed before starting the calibration process. Measurement settings should be selected in the network analyzer. Temperature of distilled water is measured using thermometer and entered in the software. Measurements were performed between 500 MHz to 18 GHz with 250 MHz intervals.

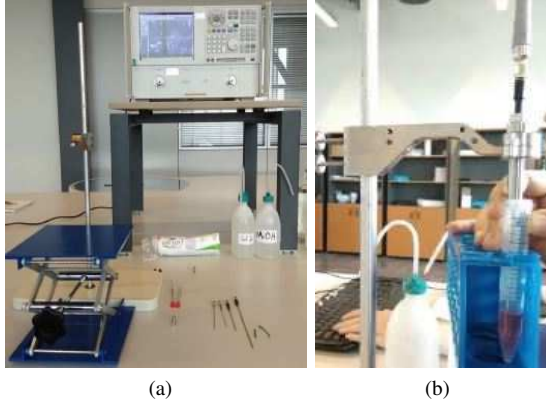


Fig. 1. Dielectric property measurement set-up: (a) Vector Network Analyzer (VNA) and measurement stand, (b) measurement sample and the open-ended coaxial probe.

B. Measurement Samples

The measurement samples are obtained from Istanbul Technical University, Department of Molecular Biology and Genetics. To prepare the cells, standard adherent cell culture protocol has been applied. They were cultured in DMEM which was supplemented with 10% fetal bovine serum (FBS), and 1% Penicillin-Streptomycin and maintained at 37°C CO₂ (5%) incubator. Cells were passaged using trypsin when confluency reached approximately 80%. SaOS-2 cells were first plated in 25 cm² cell culture flask, then they were transferred into a 75 cm² flask and finally into two 75 cm² flasks to obtain high cell number. Media was changed every two days. The flask was observed under microscope to confirm the cells were detached from the flask. After 90% of cells have detached,

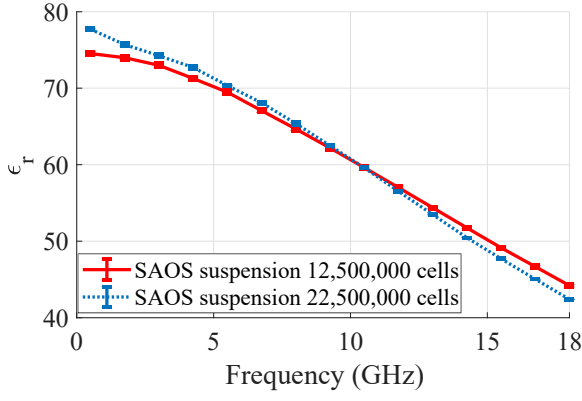
4 ml of complete growth medium was added to flask and cells were collected into 15 ml falcon tube and centrifuged for 5 minutes at 1000 rpm. After centrifugation, supernatant was discarded, and pellet was re-suspended with a minimal volume of medium without serum. A sample was taken and dyed with equal volume of Trypan Blue for counting. The sample was placed into hemocytometer and observed under microscope. Cell counting is based on the principle that living cells exclude the Trypan Blue dye and appear white while dead cells take up the dye and appear blue. The number of cells for the first sample was determined as 12,500,000 and the number of cells for the second sample was 22,500,000. After cells were counted, they were centrifuged again at 1000 rpm for 1 minute to collect the cells. Obtained cell pellets were then suspended with 3 ml medium without serum to prepare the suspension.

III. RESULTS

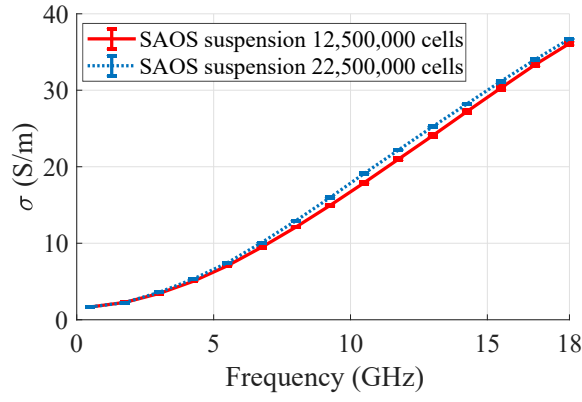
Two experiments have been done with cancer cell line (SaOS-2). In the first experiment, samples were prepared as containing 12,500,000 cells/ml, and in the second experiment sample contained 22,500,000 cells/ml. The aim was to compare the effect of the number of cells in the solution to the dielectric properties. We compared the obtained results with the data in the literature. The relative permittivity and conductivity measurements of two different concentrations are given in Fig. 2a and Fig. 2b.

The relative permittivity of high concentration solution is higher at lower frequencies and at high frequencies the relative permittivity is lower. From this we can interpret that the system uncertainty does not allow us to quantify the dielectric property discrepancy between the concentrations well. It should be noted that the two experiments are performed independently in different sessions. Although the conditions were very similar the slight changes such as a slight shift in calibration can effect the measurements. To understand the effect of suspended cells, the dielectric properties of pure DMEM is compared with 12,500,000 cells in suspension. The measurement results for relative permittivity and conductivity are shown in Fig. 3a and Fig. 3b, respectively. The media relative permittivity of suspension is approximately 0.5 units lower than the dielectric properties of DMEM. This discrepancy is more significant at lower frequencies.

Finally, from these measurements it can be inferred that although the open-ended coaxial probe method has many advantages the system uncertainty is larger than the discrepancy between the dielectric properties of cell concentrations. One option is to repeat the experiments under same conditions and collect as many measurement as possible. However, the growth of the cells depend on many other factors so therefore another option is to work with more cells and use regression to estimate the dielectric properties at points in between. It should also be noted that the volume of the cell pellet is very small despite the large number difference between two suspensions, therefore suspension can be prepared within a lower DMEM volume eg. 1 ml DMEM can be used instead of 3 ml DMEM to



(a)



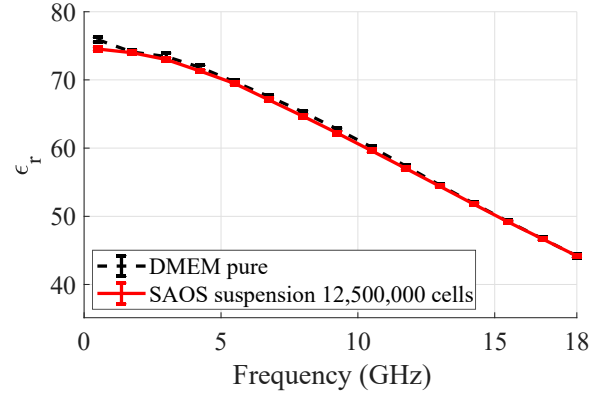
(b)

Fig. 2. Effect of number of cells to measured dielectric properties: (a) comparison of permittivity of SAOS-2 cell pellet including 12,500,000 cells/ml and 22,500,000 cells/ml measured with 2.2 mm probe, (b) comparison of conductivity of SAOS-2 cell suspension in DMEM including 12,500,000 cells/ml and 22,500,000 cells/ml measured with 2.2 mm probe.

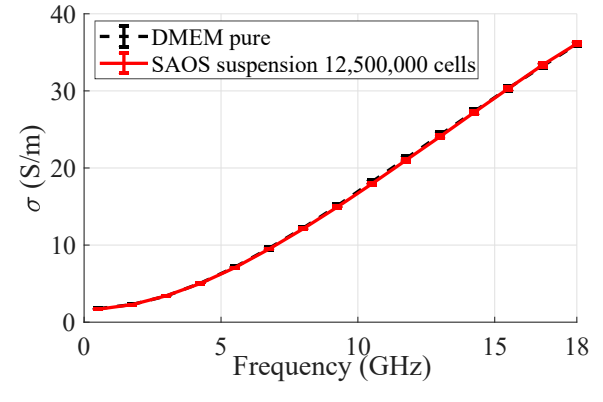
increase the concentration. While doing so, the sensing depth of the utilized probe should also be considered.

IV. CONCLUSIONS

The dielectric properties of pure DMEM and cells suspended in DMEM in different concentrations have been reported. The dielectric properties are collected with the open-ended coaxial probe technique between 500 MHz to 18 GHz. The measurements are performed with Agilent's slim form probe with 2.2 mm aperture diameter. It was expected that the dielectric properties of DMEM is larger than DMEM with SAOS-2 cell suspension at all frequencies and it was observed that the cell suspension is decreasing the relative permittivity 0.5 units on average. However, a consistent decrease have not been observed between suspensions with 12,500,000 and 22,500,000 SAOS-2 cell suspensions. We conclude that the suspension should be prepared with smaller DMEM volume and the cell numbers should be increased to quantify the discrepancy between different suspension concentrations. UI-



(a)



(b)

Fig. 3. Effect of SAOS-2 cells to dielectric properties of DMEM: (a) comparison of permittivity of SAOS-2 cell pellet including 12,500,000 cells/ml and DMEM measured with 2.2 mm probe, (b) comparison of conductivity of SAOS-2 cell suspension in DMEM including 12,500,000 cells/ml and DMEM measured with 2.2 mm probe.

timately, the cells dies very quickly under stress. This factor should also be considered while measuring cell suspensions with different concentrations.

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